Monitoring of Selected Herbicides, Antibiotics, Steroids, and Industrial Chemicals in Water by ELISA

Fernando Rubio, Abraxis LLC
Kristy Ramsey, Abraxis LLC
Paul Stackelberg, USGS
Michael Meyer, USGS

Organic Water Contaminants

- Greater Than 90,000 compounds in commerce
- Some manufactured and used in large quantities
- *Examples:* agrochemicals, pharmaceuticals, surfactants, flame retardants, personal care products, commercial and industrial products,
- Usage has increased over the years
- Enter water sources by a variety of environmental pathways

TARGETED COMPOUNDS

- **Herbicides**
  - Acetochlor
  - Atrazine
- **Steroid Hormones**
  - Estradiol (E2)
  - Ethenyl Estradiol (EE2)
  - Estrone (E1)
- **Antibiotics**
  - Sulfamethazine
- **Surfactants**
  - Alkylphenols
  - Alkylphenol ethoxylates
  - Alkyl ethoxylates
- **Industrial Chemicals**
  - Bisphenol A

Description of Water Samples

DWT Facility-NJ

- Urban drainage basin serving ~ 850,000 people
- >50 STPs discharge effluent to 2 streams from which DWT withdraws raw water
- Conventional treatment:
  1) Screening
  2) Addition of powdered AC
  3) Addition of acid/base, pH
  4) Coagulation
  5) Flocculation
  6) Sedimentation
  7) Primary Desinfection
  8) Filtration
  9) Secondary Desinfection
  10) Add caustic soda, pH

- **Sampling Methods**

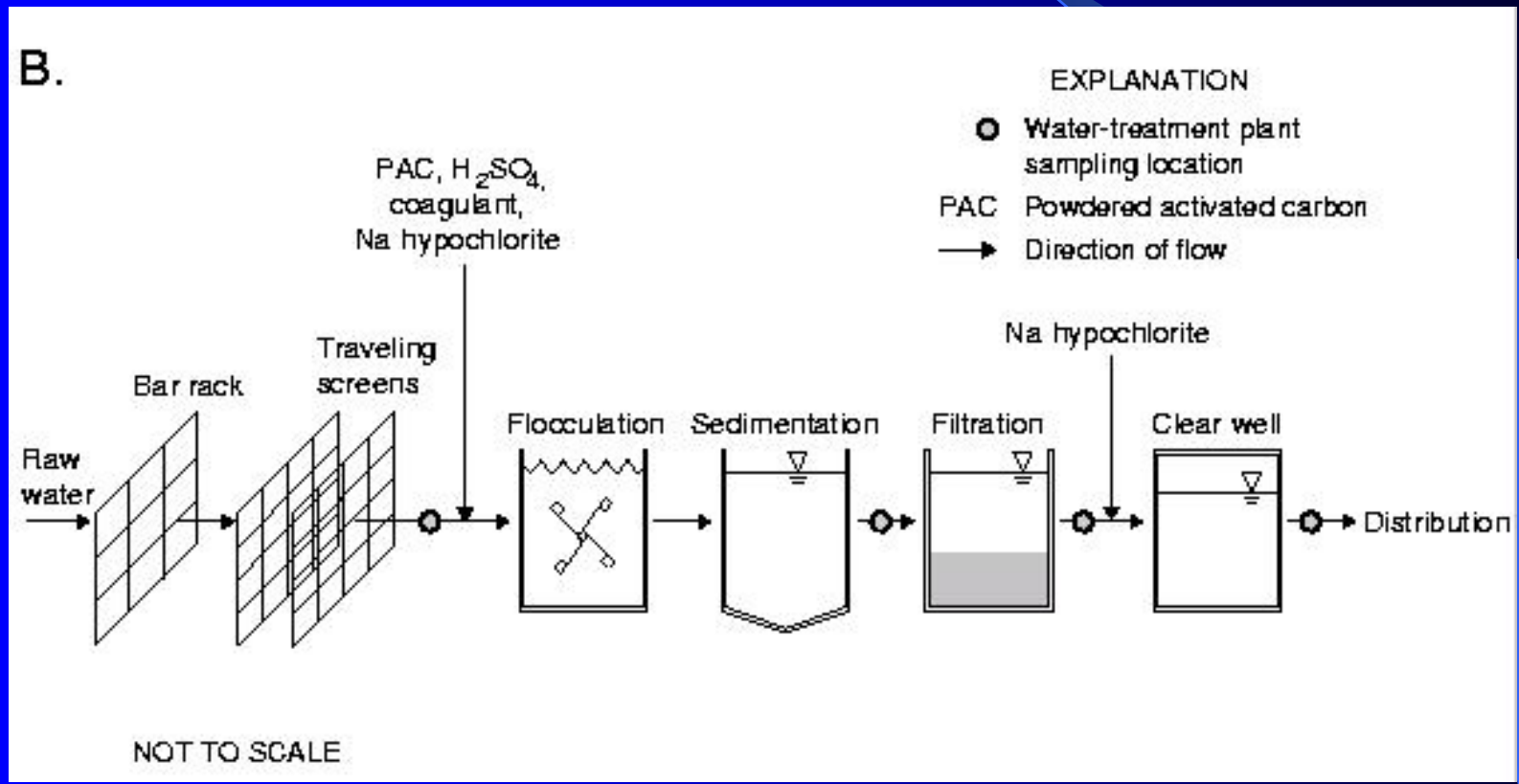
Twelve water samples collected

Over a 3 week period (July-Aug '03)

Six locations in the facility

1) Raw
2) Raw and recycled
3) Settled
4) Disinfected
5) Filtered
6) Finished

DWT Facility-NJ



Waste Treatment Plant-FL

Purpose of the Project

To determine variation of pharmaceuticals, antibiotics, household chemicals, hormones, and industrial waste products going in and coming out of the plan during low and high flow in the wet and dry season.

ANALYTICAL METHODS

- Very sensitive methods required sensitivities down to ng/ml, pg/mL)
- HPLC
- LC-MS
- GC-MS
- Immunoassay (ELISA)

Immunoassay

An immunoassay is an analytical method which uses antibodies as reagents to quantitate specific analytes

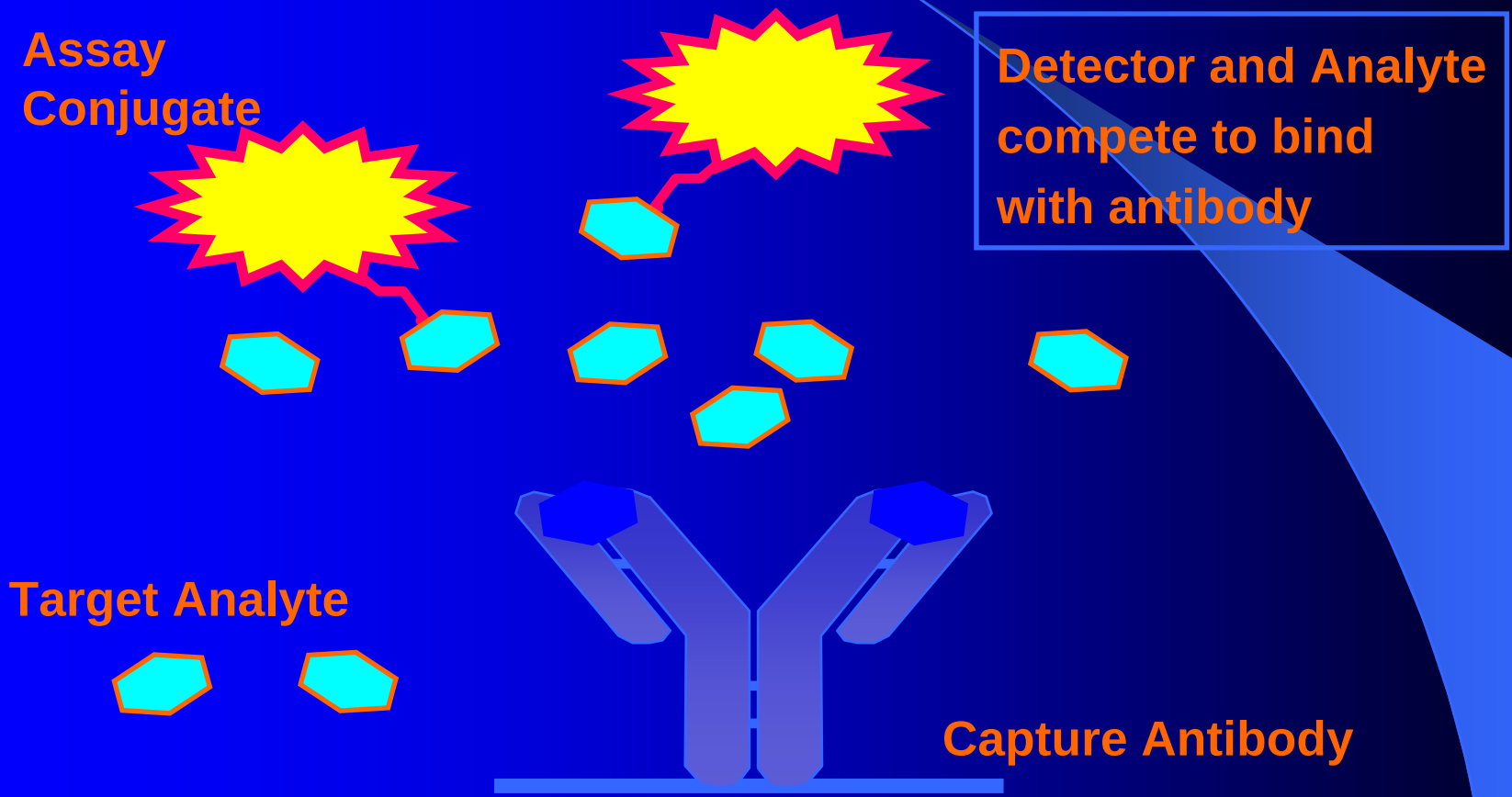
Immunoassay

- $6 Billion Industry Worldwide
- 2.5 Billion Tests Sold Annually
- Highly Quantitative
- Regulatory Approved
- Flexible Test Formats
- Diverse Markets and Applications

Immunoassay Markets

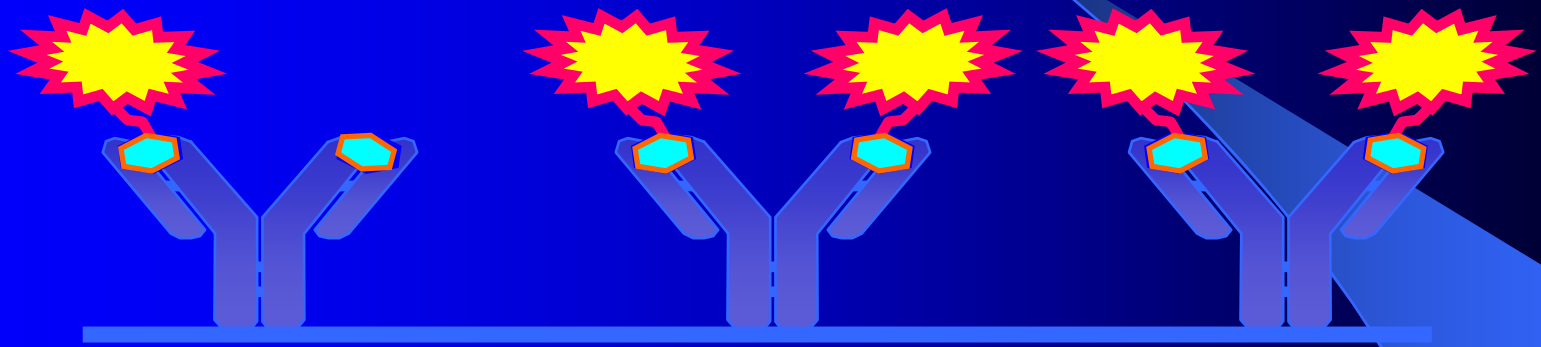
- Clinical
- Agricultural
- Environmental
- Food
- Industrial
- Pharmaceutical
- Veterinary
- Water Quality

Competitive ELISA

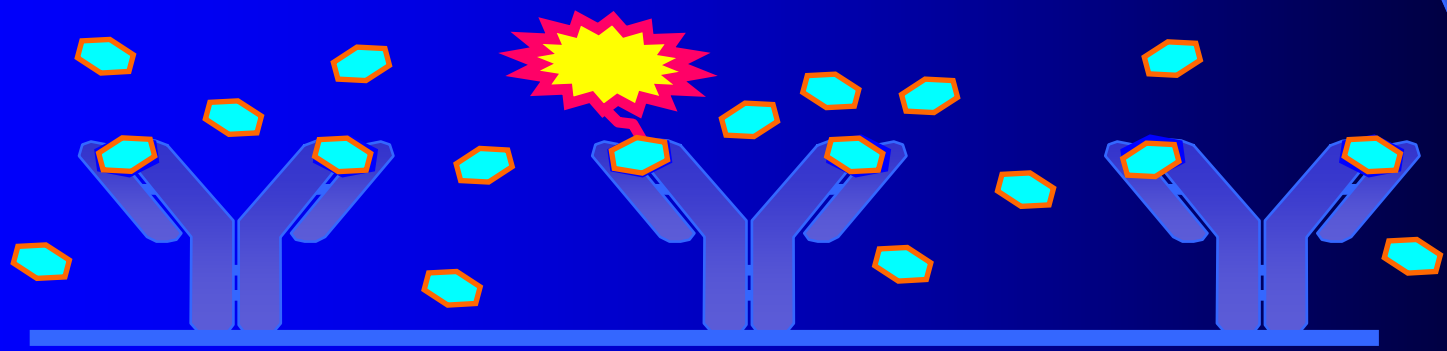


Competitive ELISA

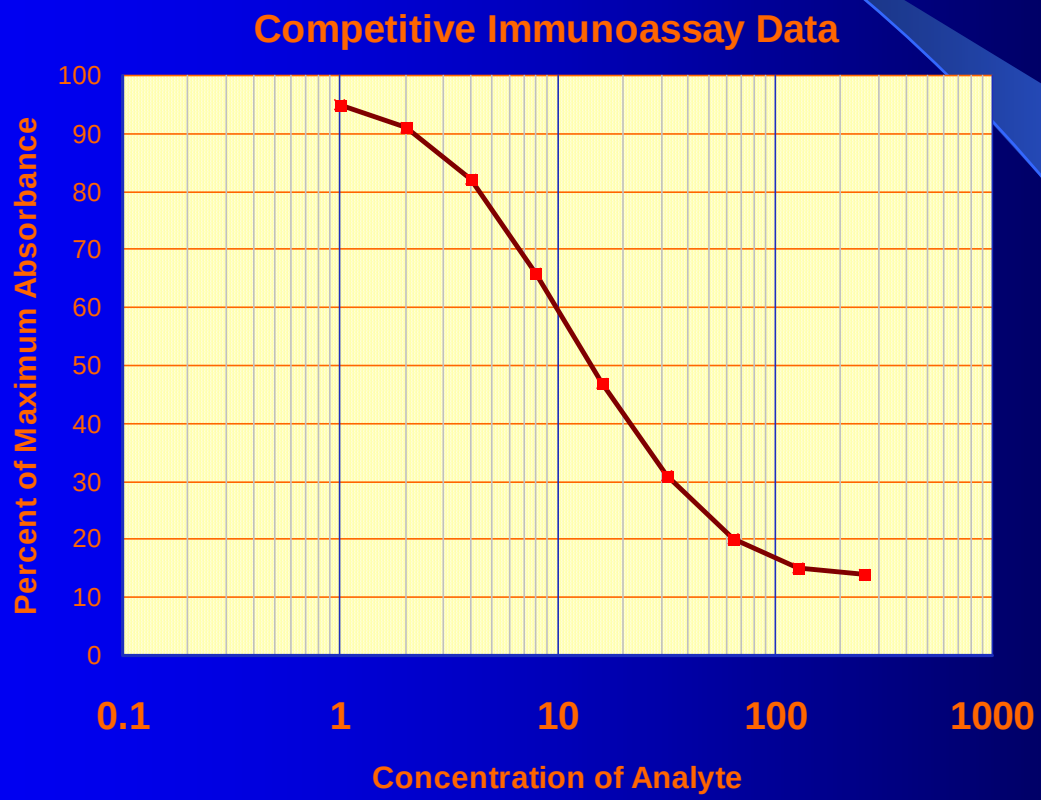
I. No analyte - high detection signal



II. Analyte present - detection signal reduced

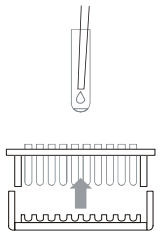


Competitive ELISA Curve



ATRAZINE CONCISE FLOWCHART

1.



Separate the rack.

Add 200 or 250 µL of either Standards, Control or Samples to the bottom of each test tube.

NOTE: Same chosen volume should be added for standards and samples.

2.

Add 250 µL of Atrazine Enzyme Conjugate to each test tube.

3.

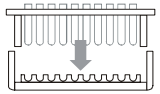
Add 500 µL of mixed Magnetic Particles to each test test tube.

Vortex.

4.

Incubate for 15 minutes.

5.

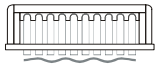


Combine the rack and magnetic base.

Seat all tubes.

Wait 2 minutes.

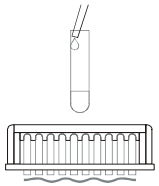
6.



Invert the combined rack.

Blot **gently**.

7.



Add 1 mL of Washing Solution.

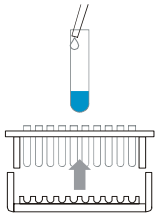
Wait 2 minutes.

Invert the combined rack.

Blot **gently**.

Repeat this step.

8.



Separate the rack.

Add 500 µL of Color Reagent to each test tube.

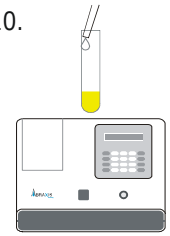
Vortex.

9.

Incubate for 20 minutes.

Prepare blank.

10.



Add 500 µL of Stopping Solution to each test tube.

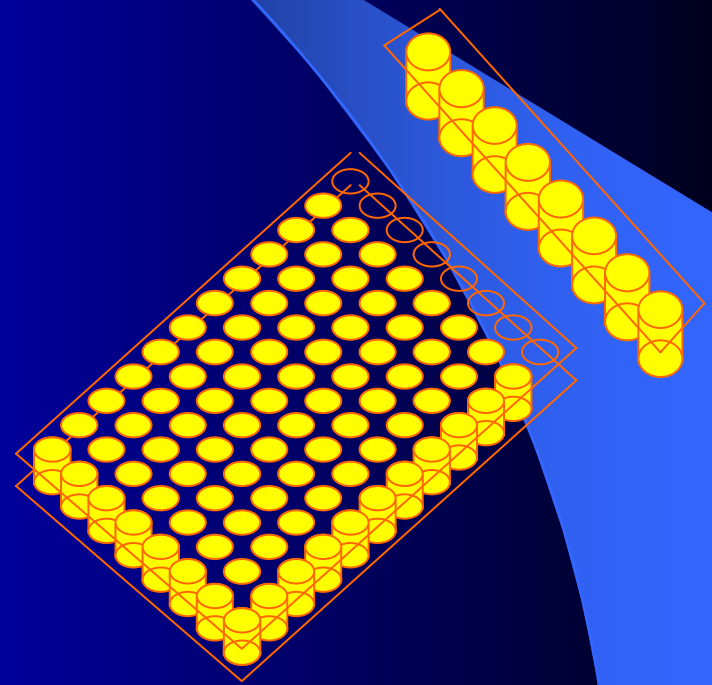
Read OD 450

For Ordering or Technical Assistance Contact:
ABRAXIS, LLC 54 Steamwhistle Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232

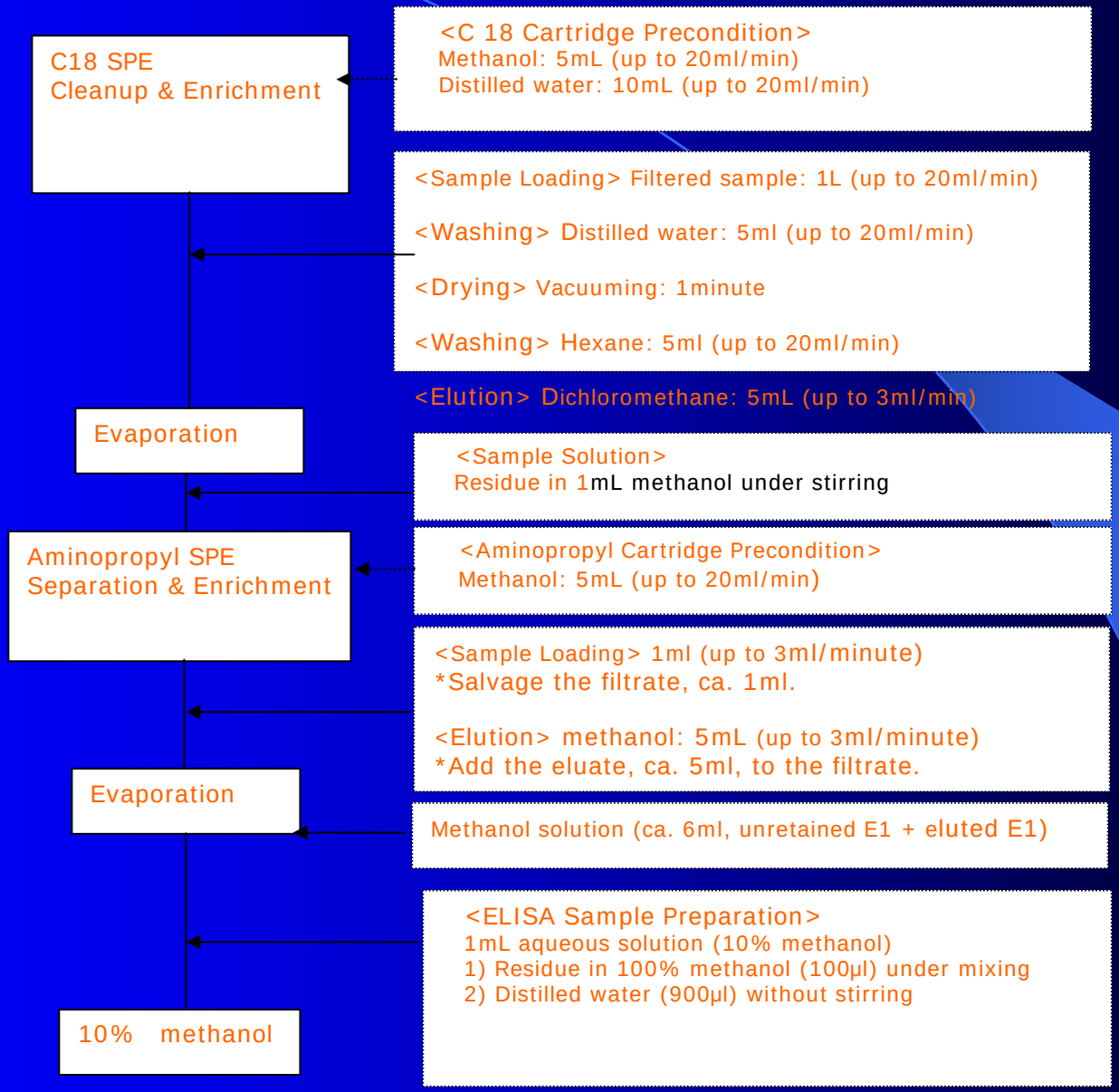
Atrazine Magnetic Particle Kit Part # 500001, 100 Test

ABRAXIS

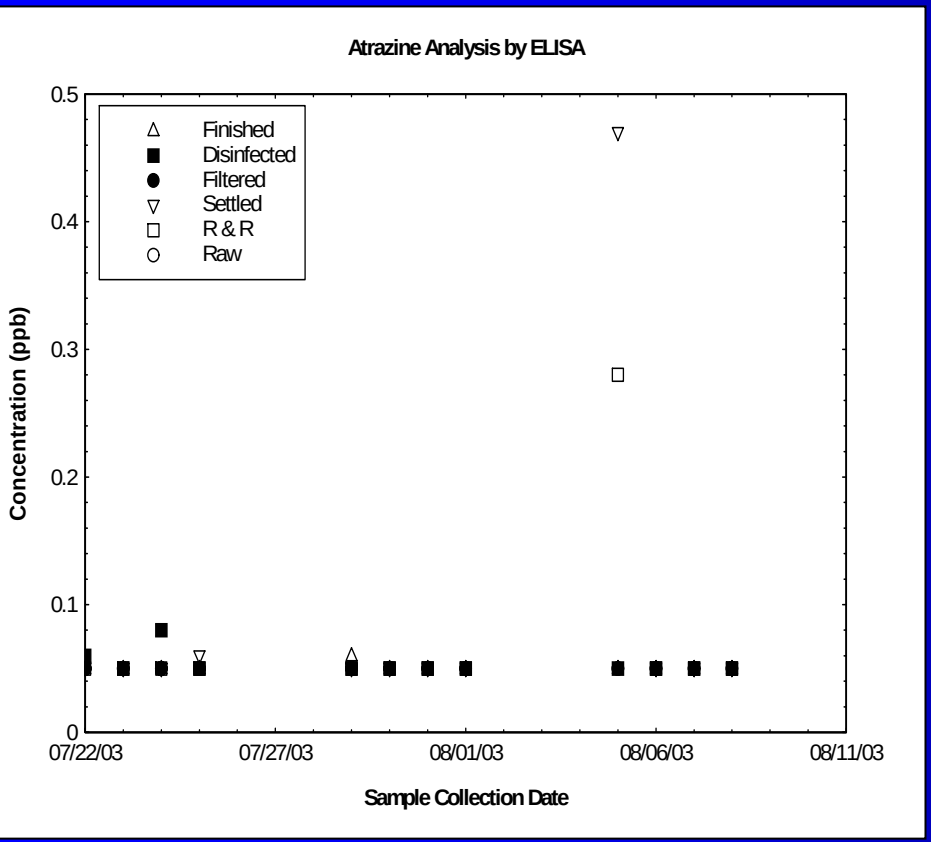
Microtiter Plate

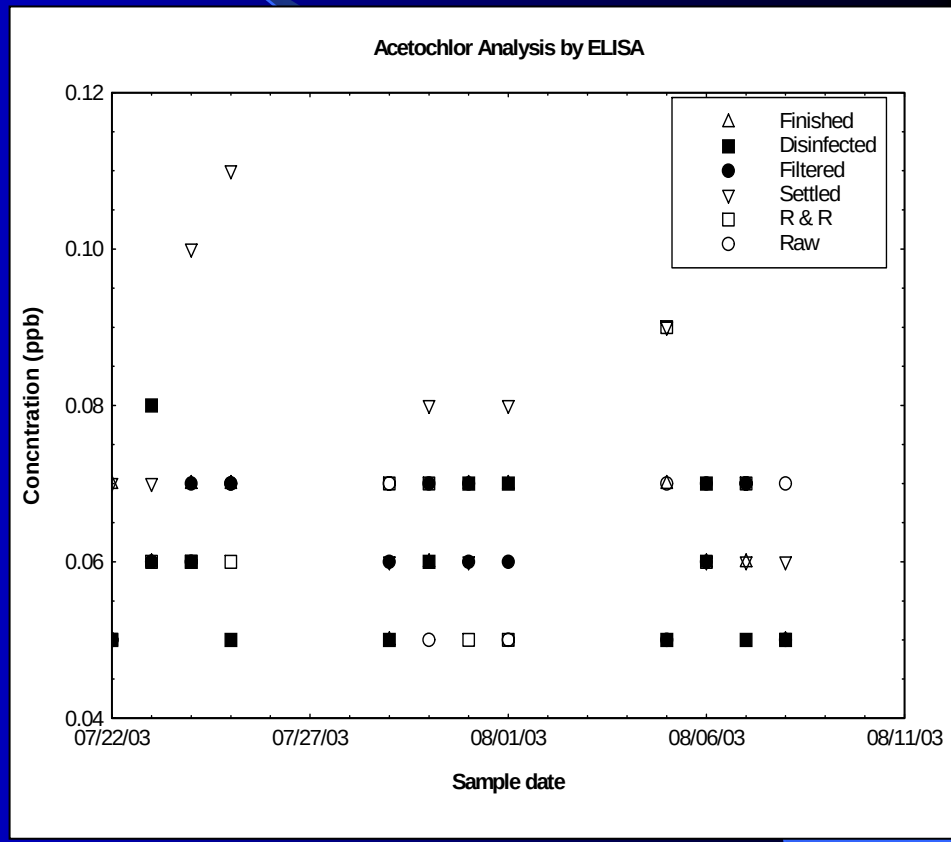


SPE Procedure, Hormones

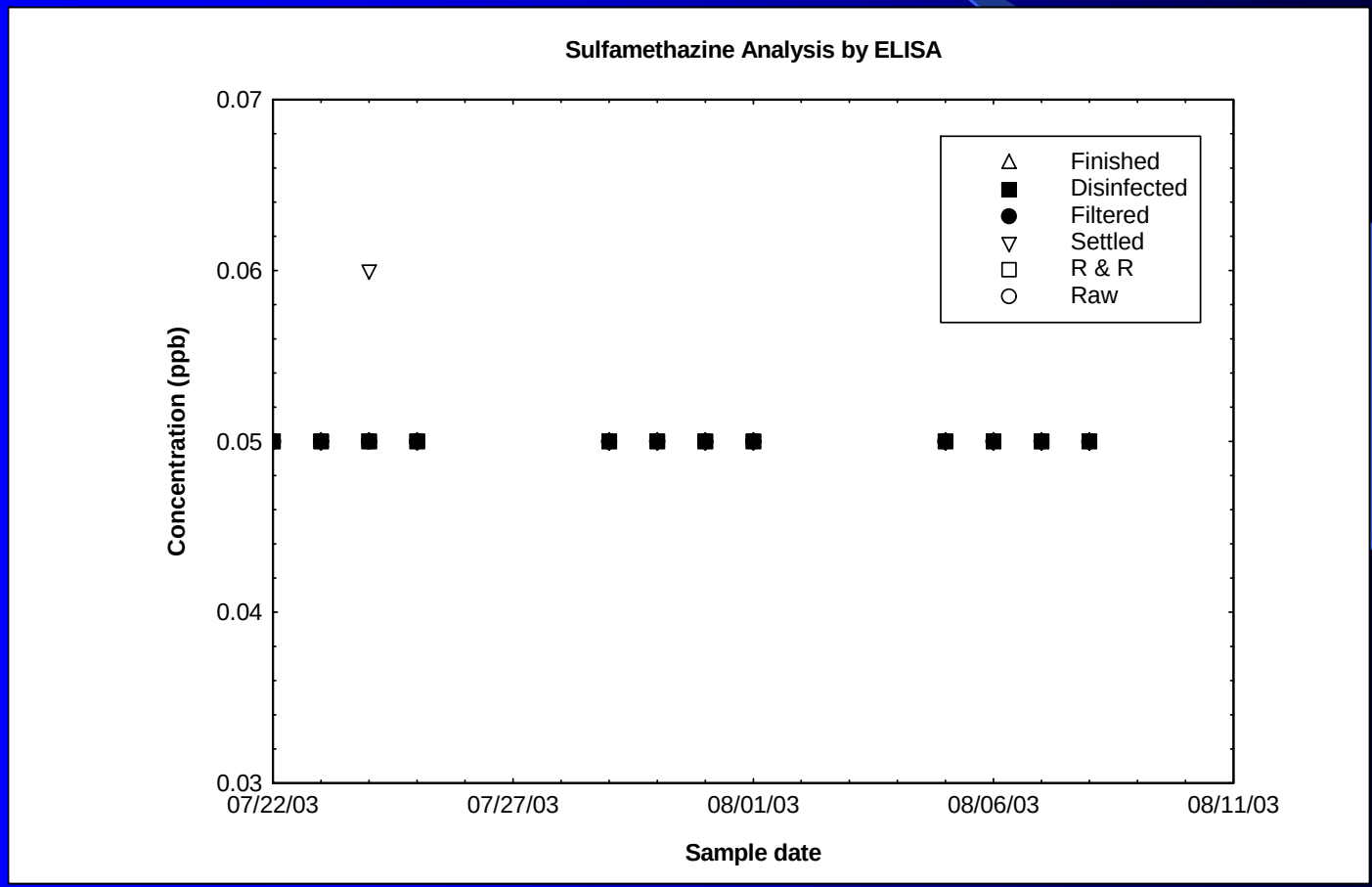


Herbicide Analysis by ELISA, NJ Samples

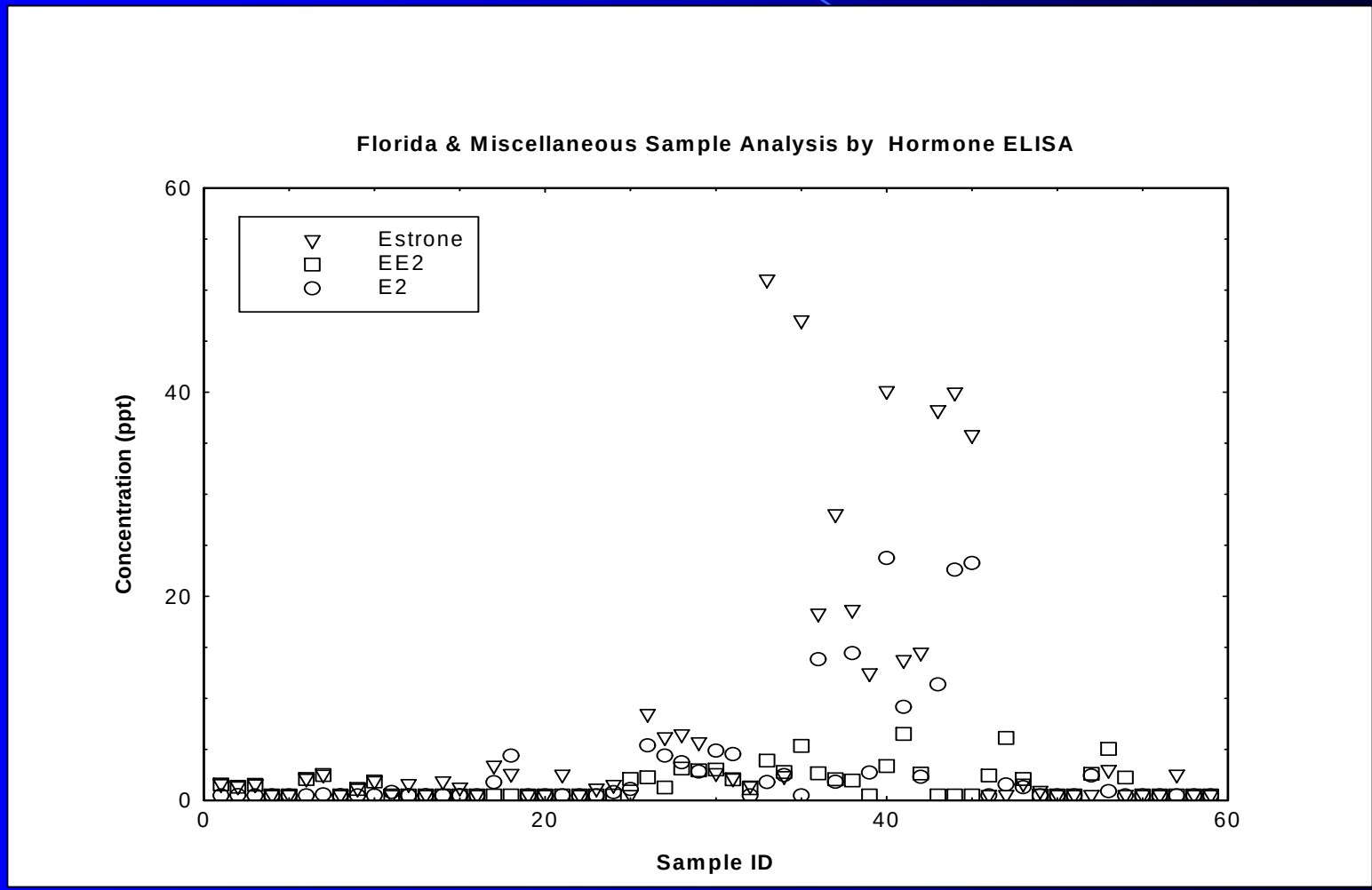




Sulfamethazine Analysis by ELISA, NJ Samples



E1, E2, EE2 Analysis by ELISA, FL Samples



E2 ELISA vs. GC, CO Samples

Sample ID	E2, ELISA, (ppt)	E2, GC (ppt)	EE2, ELISA (ppt)	E1, ELISA (ppt)
625A	<0.5	<0.8	1.2	1.4
626A	1.8	2.9	3.9	>50
627A	2.5	2.1	2.8	2.3
628A	<0.5	1.2	5.3	47
629A	13.8	1.4	2.7	18.3
630A	1.8	1.2	2.1	28

Estradiol Microtiter Plate ELISA

Atrazine Magnetic Particle Reagents

Conclusion

- ELISA Technologies, can be used to screen large number of samples timely and cost-effectively.
- Can be use for screening and for quantitation of analytes in water monitoring programs and to efficiently monitor the various stages of treatment at DWT facilities.
- Additional work is currently being performed with surfactants, bisphenol A, as well as comparison to GC values.

Acknowledgements

- Dr. Larry Barber, USGS Colorado
- Dr. James Gray, USGS Colorado

- Disclaimer: Mention of a particular product during this presentation does not constitute an endorsement by the USGS.